REMARKS

The inventors have made a significant contribution to the field of stem cell research by identifying a marker of an early liver stem cell. The stem cell is distinguished from and can be separated from other undifferentiated or partially differentiated cells in the liver by virtue of its tight association with a hepatocyte. Thus, a distinguishing characteristic of a pre-oval stem cell is that the stem cell is attached to a hepatocyte by a desmosomal junction. Further characterization revealed that the stem cell lacks expression of oval cell marker OC2, but expresses OV6, i.e., it is a pre-oval cell. The methods utilize this unique phenotype to separate cell clusters (e.g., cell doublets containing an $OC2^-$ cell) from primary liver tissue.

Claims 35-41 and 49-64 are pending. Non-elected claims 1-34 and 42-48 were canceled. Claim 35 was amended; the amendment is supported by disclosure at page 2, lines 15-35 of the specification. New claims 49-64 were added. Claim 49 is supported by disclosure at page 2, lines 32-35, and page 9, lines 1-7, of the specification. Calims 50-52 are supported by disclosure at page 3, lines 12-14, of the specification. Claims 53-55 are supported by disclosure at page 9, lines 16-23, of the specification. Claim 56 is supported by disclosure at page 2, lines 24-26, of the specification. Claim 57 is supported by disclosure at page 7, lines 29-33, of the specification. Claims 58-64 are supported by disclosure at page 4, line 26, to page 5, line 3, of the specification.

The specification was amended to recite priority information and to add an abstract.

No new matter has been added by this amendment.

35 U.S.C. § 112

Claims 35-41 were rejected for indefiniteness (lack of antecedent basis) due to the recitation of "said doublet" in claim 35. This rejection has been met by amending claim 35 to delete "said doublet".

35 U.S.C. § 102

Claims 35-38 were rejected for anticipation by Reid et al.

Reid et al. describes methods for isolating and culturing liver progenitor cells. The first step of Reid's method is to dissociate an excised section of liver into single dissociated cells.

The claims have been amended to isolate a cell cluster consisting essentially of between 2 and 5 cells in which a stem cell is attached to a hepatocyte, e.g., a cell doublet of a stem cell and a hepatocyte. The ameneded claim does not read on a section of liver tissue, nor does it read on a single cell suspension as described by Reid et al.

In view of this amendment, Applicants submit that the amended claims are novel over Reid et al.

35 U.S.C. § 103

Claims 39-41 were rejected for obviousness over Reid et al. in view of Sell and Alison. In support of this rejection, the Examiner states:

> In light of the specific suggestion of Reid et al. to use antibodies it would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to use the specific cell markers and antibodies for these markers as taught in Sell and Alison in methods to obtaine a more purified population of liver stem cells. One having ordinary skill in the art would have been motivated to use the specific cell surface markers and antibodies taught in Sell and Alison in the methodology suggested by Reid et al.

As was discussed above, the claims have been amended to require a cell cluster of 2-5 cell in which a stem cell is attached to a hepatocyte, with the stem cell having an OV6 marker

but not an OC2 marker. Applicant has defined a new phenotypic marker of an early liver stem cell (a pre-oval cell), i.e., its tight demosomal attachment to a hepatocyte. The method exploits this discovery to obtain stem cells from primary tissue. The claimed methods are the only way to distinguish this unique stem cell.

Both Sell and Alison describe oval cells, which are characterized by a panel of markers (e.g., oval cell markers OV6, OC2, and OC3, as described by Alison on page 711, column 2, Table 1). Prior to the invention, most stem cell isolation methods were based on expression of oval cell antigens. However, many of the markers described by Sell and/or Allison are present on other liver cell types (see page 8, lines 3-14, of the specification). Thus, use of such markers results in contamination of a preparation of stem cells with other cell types such as bile duct cells or mesothelial cells. The claimed methods solve the problem of contamination by requiring isolation of a specific type of liver cell cluster – one characterized by a hepatocyte joined to a small non-hepatocytic cell. Therefore, not only does this combination of references fail to suggest the invention, it does not describe a method that would yield the desired cell.

Although both Sell and Alison discuss an "even less mature periportal liver stem cell" (Sell, Abstract), neither provides any clue as to how to identify, much less isolate such a cell. Applicant has made a breakthrough in the field of liver stem cell research. He has discovered a way to identify and target a pre-oval cell, a cell that was previously elusive to researchers in the field of liver stem cells. Only by first isolating a small cluster, e.g., a doublet, is one able to identify and obtain a substantially pure population of pre-oval cells. No combination of antibodies or other markers have been able to identify and obtain such a cell from a single cell suspension. Moreover, neither Sell, nor Alison has described a marker phenotype now required by the claims: OV6+, OC2-.

In view of the foregoing amendment and arguments, Applicant submits that the amended claims are non-obvious over the combination of cited art. Withdrawal of this rejection is respectfully requested.

CONCLUSION

On the basis of the foregoing amendments and remarks, Applicant respectfully submits that the pending claims are in condition for allowance.

Applicants file concurrently herewith a petition for a two (3) month extension of time, together with a check for $645.00 to cover the fee pursuant to 37 C.F.R. § 1.17(a)(3). With the extension, this amendment is due on or before March 27, 2003. The Commissioner is hereby authorized to charge same, or credit any overpayment, to Deposit Account No. 50-0311 (Reference No. 21486-024 NATL).

Respectfully submitted,

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EXHIBIT A

Marked up Version

Cancel claims 1-34 and 42-48. Amend claim 35. Add new claims 49-64.

35. (amended) A method of obtaining a sample of substantially pure [isolated] liver stem cells comprising,

[(a)] isolating a liver cell cluster from [normal] liver tissue, said cluster comprising a stem cell associated with a hepatocyte[;], wherein said cluster consists essentially of 2 to 5 cells and wherein said stem cell comprises an OV6 antigen and lacks an OC2 antigen

[(b) dissociating said stem cell from said hepatocyte; and

(c) removing said hepatocyte from said doublet to yield a sample of liver stem cells].

--49. (new) The method of claim 35, wherein said stem cell is a pre-oval cell.--

--50. (new) The method of claim 35, wherein said liver tissue is fetal liver tissue.

--51. (new) The method of claim 35, wherein said liver tissue is pediatric liver tissue.--

--52. (new) The method of claim 35, wherein said liver tissue is adult liver tissue.--

--53. (new) The method of claim 35, wherein said liver tissue is obtained from a mouse, rat, dog, baboon, or pig.--

--54. (new) The method of claim 35, wherein said liver tissue is obtained from a human.--

--55. (new) The method of claim 35, wherein said liver tissue is obtained from a living or deceased donor.--

--56. (new) The method of claim 35, wherein said liver tissue is normal liver tissue.--

--57. (new) The method of claim 35, wherein said cluster is isolated from said liver tissue prior to a mechanical injury or exposure to a carcinogen.--

--58. (new) The method of claim 35, wherein said sample comprises at least 60% doublets.--

--59. (new) The method of claim 35, wherein said sample comprises at least 90% doublets.--

--60. (new) The method of claim 35, wherein said sample comprises at least 99% doublets.--

--61. (new) The method of claim 35, further comprising dissociating said stem cell from said hepatocyte and removing said hepatocyte to yield an population of isolated stem cells.--

--62. (new) The method of claim 61, wherein said population comprises at least 60% stem cells.--

--63. (new) The method of claim 61, wherein said population comprises at least 90% stem cells.--

--64. (new) The method of claim 61, wherein said population comprises at least 99% stem cells.--

In the specification:

On page 1, after the title, please insert:

--This application is a national stage filing of PCT/US99/15625, filed on July 8, 1999, which claims priority to patent application U.S.S.N. 09/113,774, filed on July 10, 1998, now issued as U.S.P.N. 6,129,911.--

After page 24, insert a new page 25 containing the following paragraph.